Please cite the J.B. Lawrence Lab or publications for use of this protocol. Thanks!

DNA Hybridization

1. Prepare Probe for each coverslip sample in Eppendorf tube:
   10-15µl Cot1 DNA (Roche - stock 1 ug/ul)
   2µl Salmon sperm DNA + tRNA (Sigma stock 10ug/ul of each)
   5µl of Dig or bio labled probe (1ug/80ul concentration)(~50ng nick translated probe)

2. Air dry tubes in speed vac for 15 min or until completely dry

3. **NaOH Denaturation of cellular DNA** (0.2N NaOH- 5') (This step can be omitted if you don't need to get rid of all of the RNA):
   - Transfer coverslips from 70%ETOH storage (4°C) into a coplin jar with 10mls 70%ETOH
   - Transfer coverslips from 70%ETOH into a coplin jar with 10mls 70%ETOH and 200ul 10N NaOH, incubate at Room Temp for 5 min (this gets rid of most of the RNA).
   - Rinse cells 2 times in 70% ETOH
   - Let the cells sit in the last wash until you are ready to move on

4. **Heat Denaturation of cellular DNA**:
   - In a small beaker mix:
     7ml Formamide
     2ml H2O
     1ml 20xSSC

   - Heat formamide to75°C-80°C (microwave)

   - Incubate coverslips in hot formamide, in 80°C H2O bath, in hood - 2 min.

   - Transfer slides to cold 70% ETOH - 5 min

- Replace with cold 100% ETOH - 5 min
- Air dry coverslips

5. Resuspend dried probe (from step 1) in 10µl formamide

6. Denature probe for 10 min on the 80°C heat block

7. Add 10 µl Hybridization Buffer to probe. Use immediately.

Hybridization Buffer stock: 5mls (store 4°C)

1ml	Albumin BSA
1ml	20xSSC
1ml	H2O
2ml	50% Dextran Sulfate (Autoclaved)

8. Place total volume (20µl) on a parafilm lined glass plate

9. Place coverslips, cell side down, on to the drop of liquid

10. Cover with another sheet of parafilm, press down the outer edges to make an envelope - then incubate at 37°C, overnight in humid chamber

Washes

11. Rinse in 50% Formamide/2xSSC for 20 min at 37°C

12. Rinse in 2xSSC for 20 min at 37°C

13. Rinse in 1xSSC for 20 min at room temp on a shaker

14. Rinse in 4xSSC for 1 min at room temp

Detection

15. Add 1µl appropriate secondary antibody to 500µl 4xSSC/ 1% BSA

16. Place 50-80µl of this secondary mix on parafilm lined glass plate

17. Place slips, cells down, on top of the secondary mix

18. Cover with and seal with another piece of parafilm, wrap entire plate with tin foil (keep in dark) and incubate for 1 hour at 37°c.

Rinse

19. Rinse coverslips in 10ml of:

4x SSC - 10 min on shaker in the dark

4x SSC / 0.1% Triton – 10 min on shaker in the dark
4x SSC – 10 min on shaker in the dark

DAPI

20. Incubate in DAPI stain, 30sec-1 min, in dark

21. Rinse twice with 1xPBS

22. Mount coverslips onto slides using Vectashield (Vector Labs) mounting media and seal edges with fingernail polish.